Detection of Hyperpolarized [5-13C]-Glutamine in Brain

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Introduction

Several in vitro studies were done for understanding the distribution of transport proteins mediating amino acid homeostasis in the brain1,2. At physiological state the entry of glutamin and glutamate to the central nervous system is greatly restricted and the BBB is arranged in a manner to prevent the accumulation of nitrogen rich molecules in the brain to provide an optimal chemical environment for cerebral function. A change in neurotransmitter levels (glutamate, glutamine and GABA) was demonstrated recently in the striatum at Parkinson’s disease models using 1H MRS3. Glutamine is able to cross the BBB by facilitated diffusion, however compared to other neutral amino acids this process is slower1. Actually two different transport systems were defined in the BBB for glutamine: facilitative carriers in the luminal membrane (blood facing) and sodium-dependent transporters A and N in the abluminal membranes (brain facing)1,2. The aim of this study is to use hyperpolarized [5,13C]-glutamine for the detection of glutamine transport through the intact BBB in vivo, and to evaluate its distribution in the brain region.

Methods

[5-13C]-glutamine (3M, Cambridge Isotope Labs, USA) was whirl mixed with cesium hydroxide monohydrate (0.5M, Sigma Aldrich, USA), and then dissolved in DMSO (Sigma Aldrich, USA) as glassing agent containing OX063 radical (35mM, GE Healthcare, USA) and ProHance® (4.0 mM, Bracco Imaging, Italy) until complete dissolution4. After polarizing this mixture in a 3.35 T Hypersense DNP polarizer (Oxford Instruments, UK) for 90 min, it was dissolved in 5 ml of phosphate buffer (100mM in D2O, pH = 7.2 after dissolution). All in vivo experiments were performed on healthy Lewis rats (n=2) using 3.0 T GE HTX system equipped with a dual-tuned 1H-13C volume coil. Hyperpolarized [5,13C]-glutamine was injected via tail vein (injected dose 5ml/kg; glutamine concentration 60 mM). The brain was localized using 1H-GRE images. The dynamic acquisition of 13C spectra was started with the beginning of injection. The spectra were recorded from one axial slice with thickness = 20mm; TR= 1s; flip angle = 15°; excitation frequency centered on glutamine. The acquisition of 13C images started 5 seconds after the injection of hyperpolarized glutamine. For 13C MRI a spiral read out was used with FOV 8cm; real pixel size 5 x 5 mm², slice thickness = 20mm, TR = 1 s and a flip angle of 30°.

Results and discussion

The T1 of [5-13C]-glutamine measured at 3T in vitro was 18s. The time evolution of one exemplary spectrum recorded in the brain slice after the injection of hyperpolarized [5-13C]-glutamine is shown in Fig. 1. The main peak was assigned by its chemical shift (178 ppm) to [5-13C]-glutamine. Increased [5-13C]-glutamine signals in the brain region was detected by simple display of integrated [5-13C]-glutamine images with corresponding GRE anatomical images in all animals (Fig.2). The distribution of hyperpolarized [5,13C]-glutamine was imaged but the spatial resolution does not allow to differentiate between brain and blood. However even 30 s after the injection a sufficient glutamine signal was detected in different brain areas.

Conclusion

Glutamine can be considered as a promising candidate for future studies of neurodegenerative diseases connected with a change in neurotransmitter levels. In the future 13C-MRI might provide novel and more selective tools to study the transport systems and evaluate their regulation in vivo.

References


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